

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
7 December 2000 (07.12.2000)

PCT

(10) International Publication Number
WO 00/73495 A1

- (51) International Patent Classification⁷: C12Q 1/04
- (21) International Application Number: PCT/PT00/00005
- (22) International Filing Date: 31 May 2000 (31.05.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
102306 ✓ 31 May 1999 (31.05.1999) PT
- (71) Applicants (*for all designated States except US*): **INSTITUTO SUPERIOR DE AGRONOMIA** [PT/PT]; Tapada da Ajuda, P-1399 Lisboa (PT). **STAB-TRATAMENTO DE ÁGUAS E BIOTECNOLOGIA, LDA.** [PT/PT]; Quinta de St^a. Teresa, P-2825 Charneca da Caparica (PT).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): **LOUREIRO**, Virgílio Borges [PT/PT]; Bairro da Calçada dos Mestres, Rua 9 N^o 13, P-1070 Lisboa (PT). **GONÇALVES**, Maria da Graça Alves [PT/PT]; R. 19 De Março, N^o 6 r/c Esq^o, Portela das Padeiras, P-2000-647 Santarém (PT). **RODRIGUES**, Nuno Miguel Sousa Falcão Freire [PT/PT]; Av. do Brasil, N^o 158, 2^o Esq^o, P-1700-076 Lisboa (PT).
- (74) Agents: **FERREIRA MAGNO**, Fernando António et al.; Rua das Flores, 74 - 4^o andar, P-1200-195 Lisboa (PT).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:**
- With international search report.
 - Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: CULTURE MEDIUM FOR DETECTION OF *DEKKERA* AND *BRETTANOMYCES*

(57) Abstract: The present invention provides a generic culture medium for the detection and enumeration of yeasts belonging to the *Dekkera* and *Brettanomyces* genera and a method for the detection and enumeration of said yeasts using said culture medium. According to the invention, the method comprises adding to a base yeast culture medium, a non fermentable energy source, particularly ethanol, *p*-cumaric acid, as an aromatic compound promoting substrate, exclusively produced by said yeast genera, an acid-base indicator, particularly bromocresol green, a yeast growth inhibitor antibiotic, particularly cycloheximide, and a bacterial growth inhibiting antibiotic, particularly chloramphenicol and/or oxytetracycline. When yeasts of the genera *Dekkera* and *Brettanomyces* are cultivated in said medium, the developed colonies show a characteristic color, the culture medium color changes according a reproducible pattern, due to the decrease in pH, and a characteristic phenol-like aroma is developed, easily detectable by smell after a few days of incubation, which allows their detection and enumeration. The invention is useful in the detection and enumeration of yeasts belonging to the *Dekkera* and *Brettanomyces* genera in the food and beverage industry, allowing its inclusion in yeast identification galleries.

WO 00/73495 A1